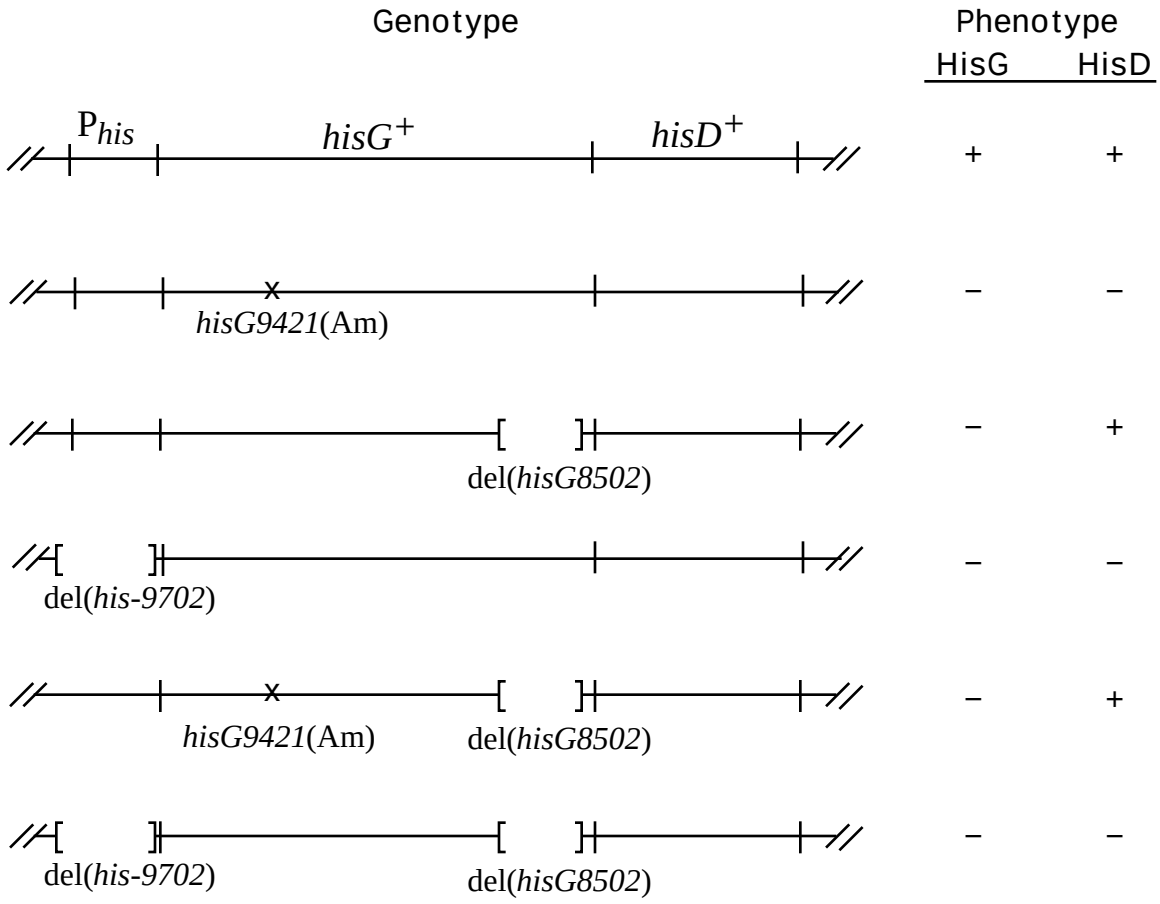
1. (10) The figure below shows the first two genes of the wild-type *his* operon and strains containing combinations of three mutations that map in the *hisG* gene.



a. What is the most likely reason why the *hisG9421*(Am) mutant is HisD$^-$?
ANSWER: Nonsense mutation results in Rho dependent transcription termination and thus is polar on *hisD* gene.

b. What is a likely reason why the double mutant *hisG9421*(Am) del(*hisG8502*) is HisD$^+$? **ANSWER: Deletion relieves Rho-dependent termination, thus transcription and subsequent translation of *hisD* gene occurs.**

c. What is the most likely reason why the del(*his-9702*) mutant is HisD$^-$? **ANSWER: Promoter is deleted thus no transcription of downstream genes.**

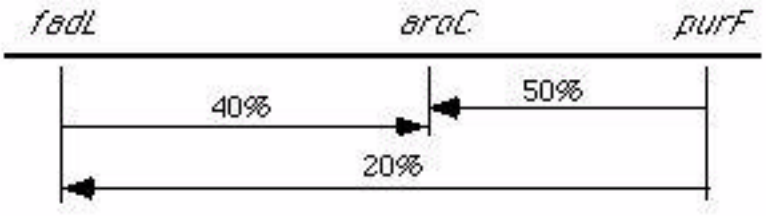
d. What is a likely reason why the double mutant del(*his-9702*) del(*hisG8502*) is HisD$^-$? **ANSWER: Promoter is deleted therefore no transcription of downstream genes whether or not the *hisG* deletion is present.**

e. What would the HisD phenotype of the del(*his-9702*) *hisG9421*(Am) del(*hisG8502*) triple mutant be? Briefly explain the rationale for your answer. **ANSWER: HisD$^-$ because promoter is deleted.**

2. (12) P22 transduction was done to map the *fadL* gene. The results of two-factor crosses between *fadL* and two linked markers, *purF* and *aroC*, are shown below: From these data, draw a linkage map of the *fadL*, *purF*, and *aroC* genes. [Indicate the predicted gene order and the percent cotransduction between each gene.]

Donor	Recipient	Selected marker	Recombinants	Number obtained
fadL purF$^+$	*fadL*$^+$ *purF*	*purF*$^+$	*fadL*	200
			fadL$^+$	800
fadL aroC$^+$	*fadL*$^+$ *aroC*	*aroC*$^+$	*fadL*	400
			fadL$^+$	600
aroC$^+$ *purF*	*aroC purF*$^+$	*aroC*$^+$	*purF*	500
			purF$^+$	500

ANSWER:



3. (12) To confirm the gene order determined from two-factor crosses, the following three factor cross was done to map *fadL*.

Donor *fadL purF*$^+$ *aroC*$^+$
Recipient *fadL*$^+$ *purF aroC*

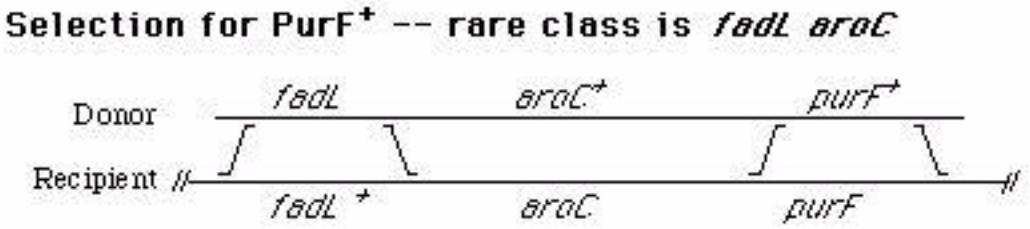
Selected phenotype	Recombinants obtained	Number
PurF$^+$	*fadL*$^+$ *aroC*$^+$	180
	fadL aroC$^+$	130
	fadL$^+$ *aroC*	240
	fadL aroC	20
AroC$^+$	*fadL*$^+$ *purF*$^+$	300
	fadL purF$^+$	250
	fadL$^+$ *purF*	330
	fadL purF	270

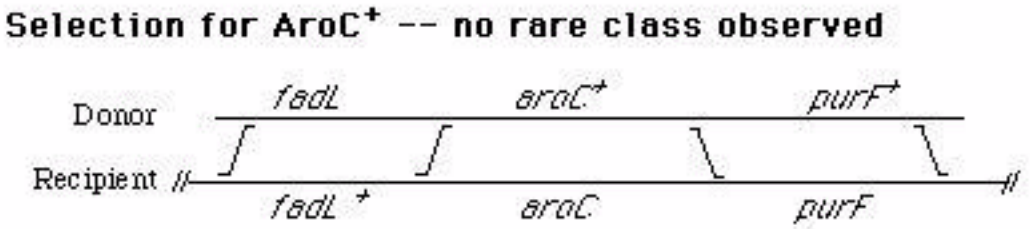
a. Based on these data, what is the order of the *fadL*, *purF*, and *aroC* genes?

ANSWER: *fadL - aroC - purF*

b. For each selected phenotype, draw the crossovers leading to this conclusion and briefly explain your rationale.

ANSWER:

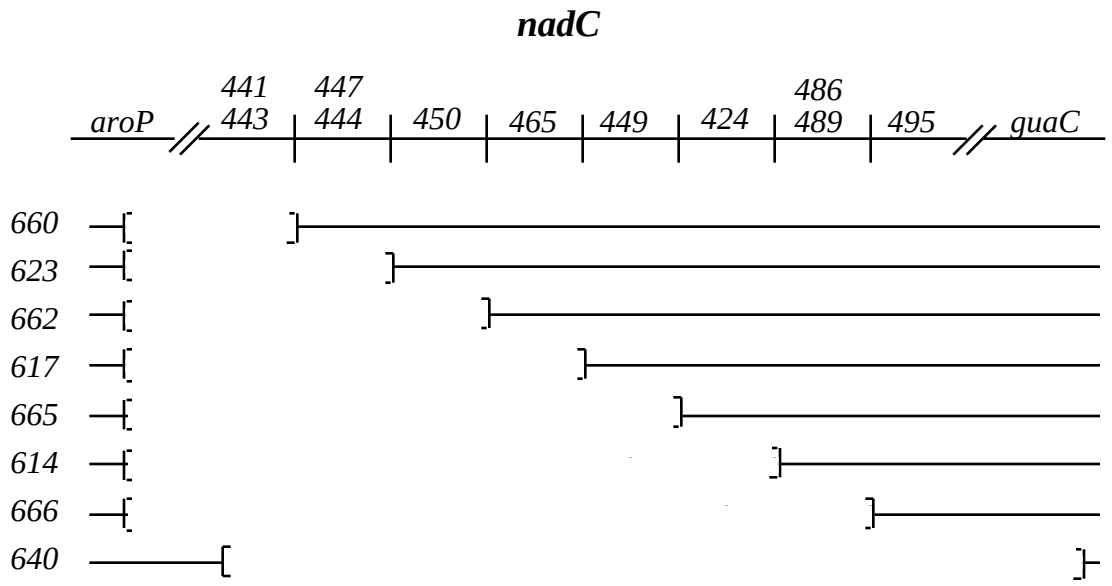




4. (12) The *nad* genes are required for the synthesis of NAD.

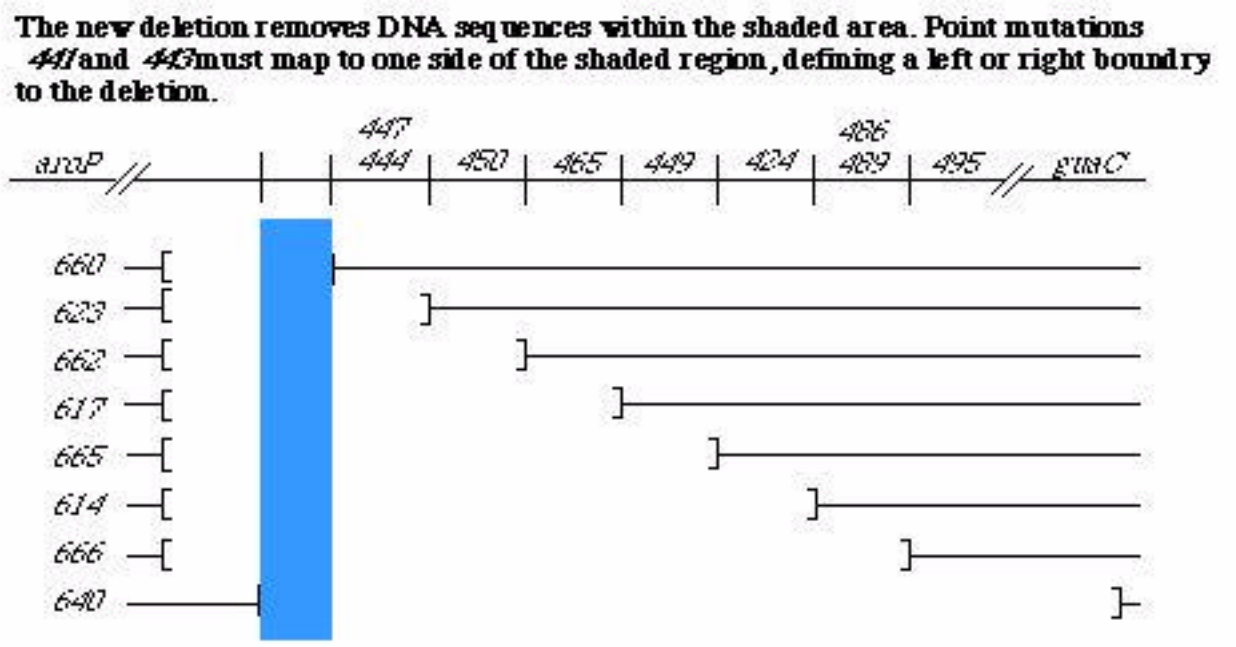
a. A new Nad$^-$ mutant was isolated. The mutation can be complemented by *nadC*$^+$ in trans, but it cannot revert to NadC$^+$. What is the simplest interpretation of these results?
ANSWER: Due to a deletion that removes at least part of the *nadC* gene.

b. To determine the location of the mutation, P22 HT was grown on the new Nad$^-$ mutant and used to transduce each of the *nadC* point mutants and deletion mutants shown on the following map. [The DNA between the brackets is deleted from the indicated strain.]



None of the transductions with *nadC* deletion mutants yielded Nad^+ colonies, but all of the transductions with *nadC* point mutants yielded Nad^+ colonies. Based upon these results, indicate the position of the mutation within the *nadC* gene. [Show the location on the deletion map and briefly explain your rationale for this conclusion.]

ANSWER:



5. (6) Recall that the *E. coli hfl* gene affects the lysis-lysogeny decision of phage λ.

a. What results would you expect if you infected an *E. coli hfl gal* mutant with λd*gal*$^+$ HFT? Why? **ANSWER: In the absence of the Hfl protease, a high concentration of cII would**

accumulate, favoring lysogeny. Thus, a high frequency of Gal$^+$ transductants would be obtained.

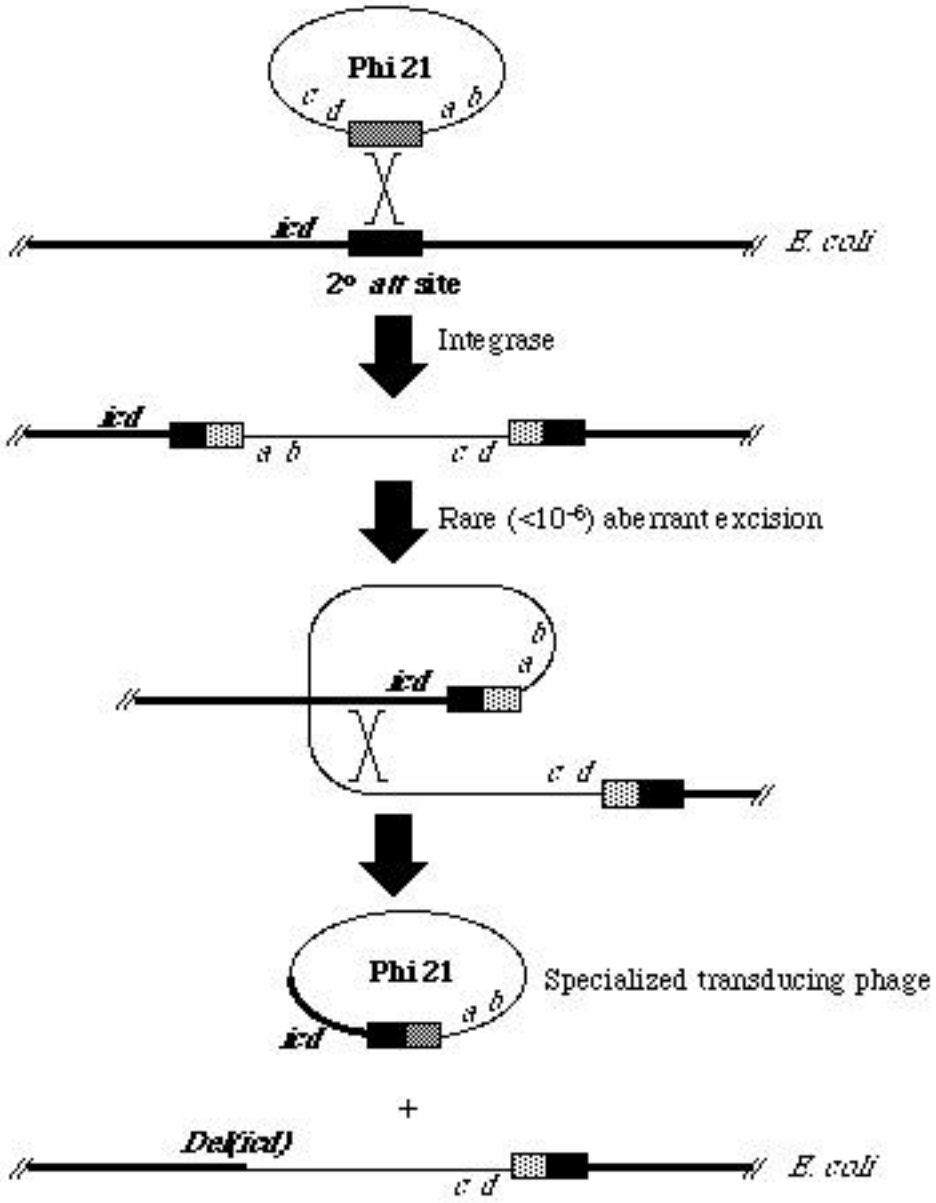
b. Would the MOI used affect your answer? Briefly explain. **ANSWER: At a very low MOI, few cells would be infected so few Gal$^+$ transductants would be obtained. However, because of the absence of the Hfl protease, the number of phage per cell will have little effect on the lysis-lysogeny decision. Note that an HFT lysate also contains wild-type phage, but these phage will also enter the lysogenic pathway.**

c. What would happen if the *E. coli hfl* mutant was already a λ lysogen? Why? **ANSWER: The cI repressor protein made by the prophage would repress the incoming phage -- no integrase would be produced so the only way the incoming phage could integrate into the chromosome would be via homologous recombination with the preexisting lysogen. Homologous recombination of the *gal*$^+$ genes on the phage with the *gal* genes on the chromosome could yield Gal$^+$ transductants, but the frequency of this would be low relative to the number obtained following site-specific recombination of the phage.**

6. (8) There is an attachment site for the lambdoid phage ø21 adjacent to the *icd* gene on the *E. coli* chromosome. *icd* mutants are glutamate auxotrophs.

a. Draw a diagram showing how a LFT containing *icd* specialized transducing particles could be formed following infection of a ø21^S host. Indicate the expected frequencies for any rare events.

ANSWER:



b. How would you test for *icd* specialized transducing particles? [Indicate the recipient you would use and the phenotype you would test.] **ANSWER: Use a Phi21^{s} recA icd recipient. Select for growth on minimal medium without glutamate. Note that the recA mutation insures that any transduction to Icd^{+} is due to specialized transduction, not generalized transduction.**

7. (6) The genes encoding cholerae toxin (*ctxAB*) can be transferred by mixing a supernatent from a virulent *Vibrio cholerae* strain with a nonvirulent *Vibrio cholerae* strain. The supernatent can transfer the *ctxAB* genes even if it has been passed through a millipore filter that excludes whole cells or if the extract has been treated with DNase.

a. What can you conclude from these results? **ANSWER: Conjugation requires cell to cell contact which is excluded by millipore filter. Transformation with naked DNA**

would be prevented by treatment with Dnase. Thus, the *ctxAB* genes are transferred by a phage. (DNA in the phage head if protected from DNase.)

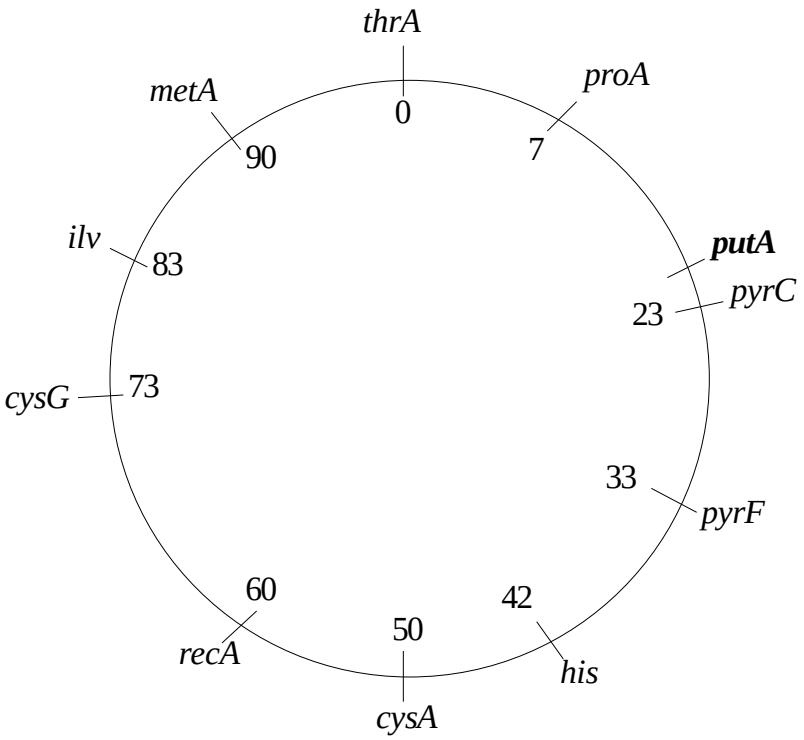
b. When transferred to recipient cells the *ctxAB* genes are found at a specific site on the *Vibrio cholerae* chromosome. What is the most likely explanation for this result? **ANSWER: The phage carrying *ctxAB* integrates at a specific attachment site on the chromosome.**

c. The *ctxAB* genes cannot be transferred to recipient *Vibrio cholerae* strains that have a mutation in the structural gene for the Tcp-pilus. What is the most likely explanation for this result? **ANSWER: The Tcp pilus is the phage receptor on the cell surface.**

8. (4) A new phage grows on *Vibrio cholerae*. It acts as a generalized transducing phage on some strains of *Vibrio cholerae* but it acts as a specialized transducing phage on other strains of *Vibrio cholerae*. How could you distinguish generalized transduction from specialized transduction using simple genetic tests? [Indicate any mutations in the donor or recipient strains you would use and how you would do the experiment.] **ANSWER: Could eliminate generalized transduction by testing for inheritance in a *recA* recipient. (Specialized transduction is RecA-independent.) Could eliminate specialized transduction by testing for inheritance of donor markers from multiple sites around the chromosome.**

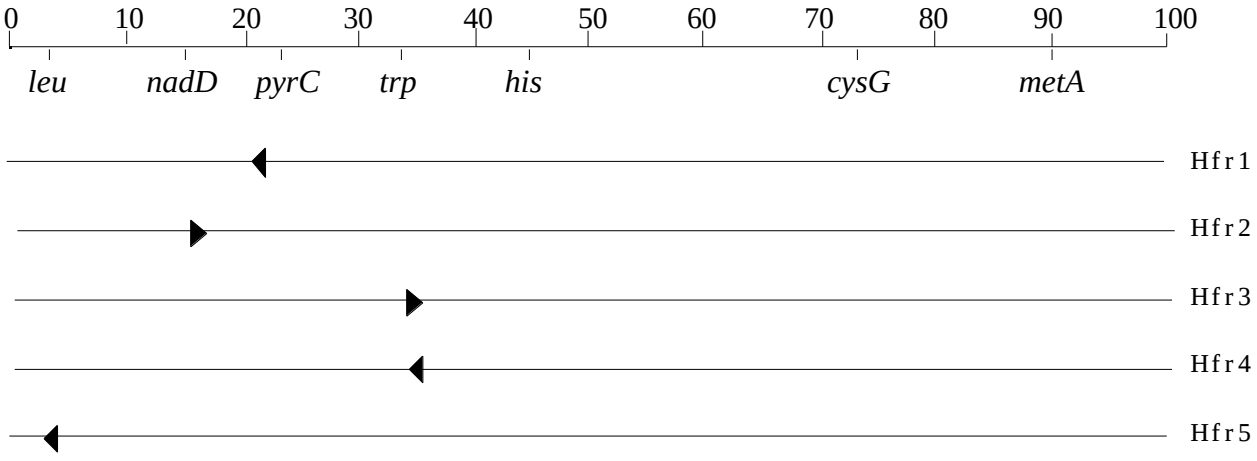
9. (4) The F-plasmid forms Hfr's at a much lower frequency in *Salmonella typhimurium* than in *Escherichia coli.* In addition, there are many fewer insertion sites in *S. typhimurium* compared to *E. coli*. Given what you know about how Hfr's are formed, what is the most likely explanation for this difference between *S. typhimurium* and *E. coli*? **ANSWER: F integrates at specific IS sequences. Probably fewer IS sequences *in S. typhimurium* than in *E. coli*.**

10. (8) A map of the *S. typhimurium* chromosome is shown below. The *putA* gene maps adjacent to the *pyrC* gene at 22 min on the genetic map. Given a Hfr insertion in the *pyrC* gene, how could you isolate a F' that carries the *putA*$^+$ gene? [Note: you do <u>not</u> know the orientation of transfer of the Hfr.] Be sure to describe any mutations in the donor or recipient needed for your experiment.



ANSWER: Because you do not know the orientation of the Hfr, you do not know whether the *putA* gene would be transferred by the Hfr early or late. Therefore, to obtain an F' that carries the *putA*$^+$ gene you could mate a Hfr *put*$^+$ *metA* donor with *a put metA*$^+$ *recA* recipient, selecting for Put$^+$ Met$^+$ transconjugants. [In this example, the counterselection was demanding methionine prototrophy, but you could also use a variety of other counterselections.]

11. (6) Wild-type*Salmonella typhimurium* LT2 is unable to use histidine as a sole nitrogen source (Hut$^-$). A *S. typhimurium* mutant was isolated that acquired the ability to use histidine as a nitrogen source (Hut$^+$). To determine where the mutation mapped, LT2 was mated with five different Hut$^+$ Met$^-$ Hfr donor strains. The *metA* mutation makes the donor cells methionine auxotrophs. The map position and orientation of each Hfr donor is shown in the figure below.



a. How could you select against the recipient cells in this experiment? **ANSWER: Hut$^+$ = medium with histidine as a sole-nitrogen source**

b. How could you counterselect against the donor cells in this experiment? **ANSWER: Met$^+$ = medium with no methionine**

c. The results are shown in the following table. Based upon these results, what is the most likely map position of the *hut*$^+$ mutation? [Indicate the map position relative to the Hfr origins.]

Donor strain	Hut$^+$ colonies
Hfr 1	0
Hfr 2	0
Hfr 3	500
Hfr 4	0
Hfr 5	450

ANSWER: Between the F-insertion of Hfr 1 and Hfr 2.

12. (12) Gardner isolated a strain of *E. coli* that was resistant to the antibiotics tetracycline (Tet^R) and kanamycin (Kan^R) but sensitive to nalidixic acid (Nal^S). To determine whether the antibiotic resistance was encoded on a conjugal plasmid, he mated this Tet^R Kan^R donor with two different Tet^S Kan^S Nal^R *E. coli* recipient s. The results are shown below. "Donor only" and "recipient only" controls are also shown.

Donor cells	**Recipient cells**	**Number of colonies on plates with:**	
		Tet + Nal	Kan + Nal
Tet^R Kan^R Nal^S	none	2	5
none	*E. coli* B Tet^S Kan^S Nal^R	0	0
Tet^R Kan^R Nal^S	*E. coli* B Tet^S Kan^S Nal^R	7	4
none	*E. coli* C Tet^S Kan^S Nal^R	0	0
Tet^R Kan^R Nal^S	*E. coli* C Tet^S Kan^S Nal^R	500	80

a. Given the results in the table, what can you conclude about the transfer of the Tet^R and Kan^R genes? [Explain your logic.] **ANSWER: Because they are transferred at different frequencies, they are probably present on different DNA molecules (e.g. separate plasmids).**

b. What is a likely reason for the difference between the results with the two different recipient strains? **ANSWER: The restriction barrier in *E. coli* B. Recall that *E. coli* C is restriction minus.**

c. Describe three ways that gene transfer might have occured under these conditions, briefly compare and contrast each of the three mechanisms, and indicate how you could experimentally distinguish between them.

ANSWER:

- **Transformation - transfer of naked DNA from lysed donor cells into recipiet cells; the DNA would be sensitive to DNase; no cell-cell contact required**
- **Transduction - transfer of DNA packaged in phage particles; the DNA would be resistant to DNase; no cell-cell contact is required and the transducing particles can pass via a filter that excludes cells**
- **Conjugation - transfer of DNA via direct cell-cell contact between donor and recipient cells; the DNA is resistant to DNase**